Optimizing Pollen Confinement in Maize for Regulated Products

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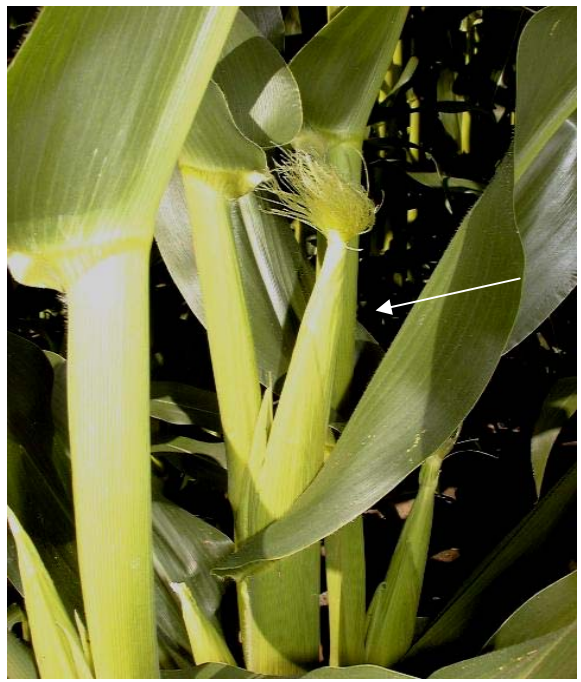
Plant-Made Pharmaceutical Corn

- Open pollination
- Controlled pollination
  - Male sterility
  - Bag tassels
  - Detasseling ✓

One PMP production system

Non-trangenic male

Detasseled transgenic female



Harvested for PMP proteins or seed increase

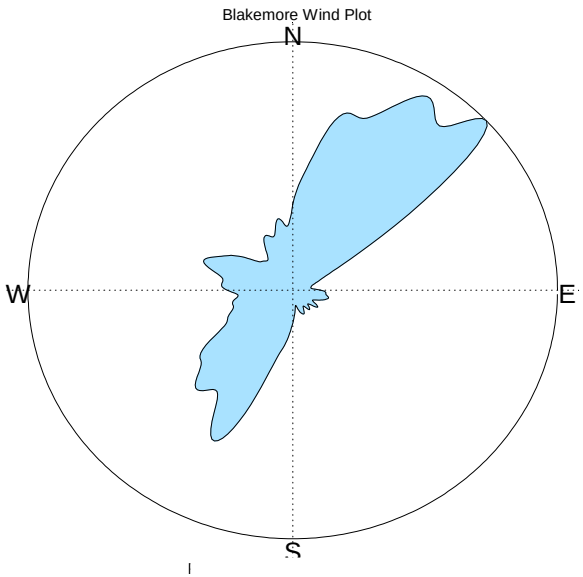
Hybrid seed from inbreds

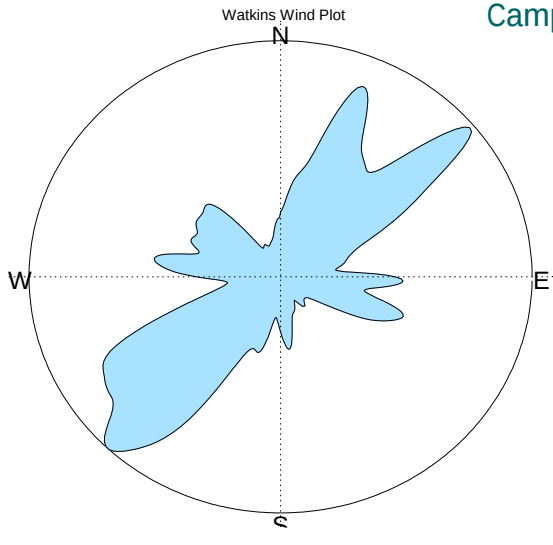
Objective

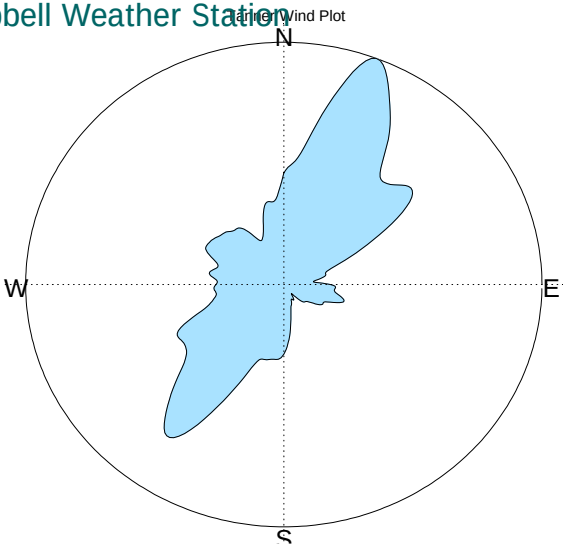
- By using maize isolines, simultaneously assess detasseling efficiency effects on gene flow with PMP confinement strategy across multiple environments.
- Results from trap plots with best pollen synchrony (“nick”).

Wind Direction 2001

Campbell Weather Station







Clarkton Hayti Wardell

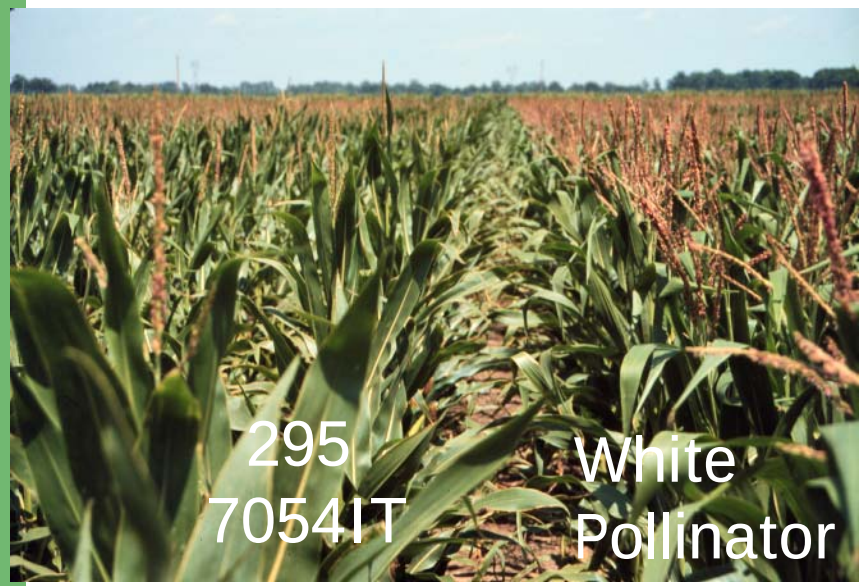
Based on wind direction and speed from 8 to 11 am during pollination

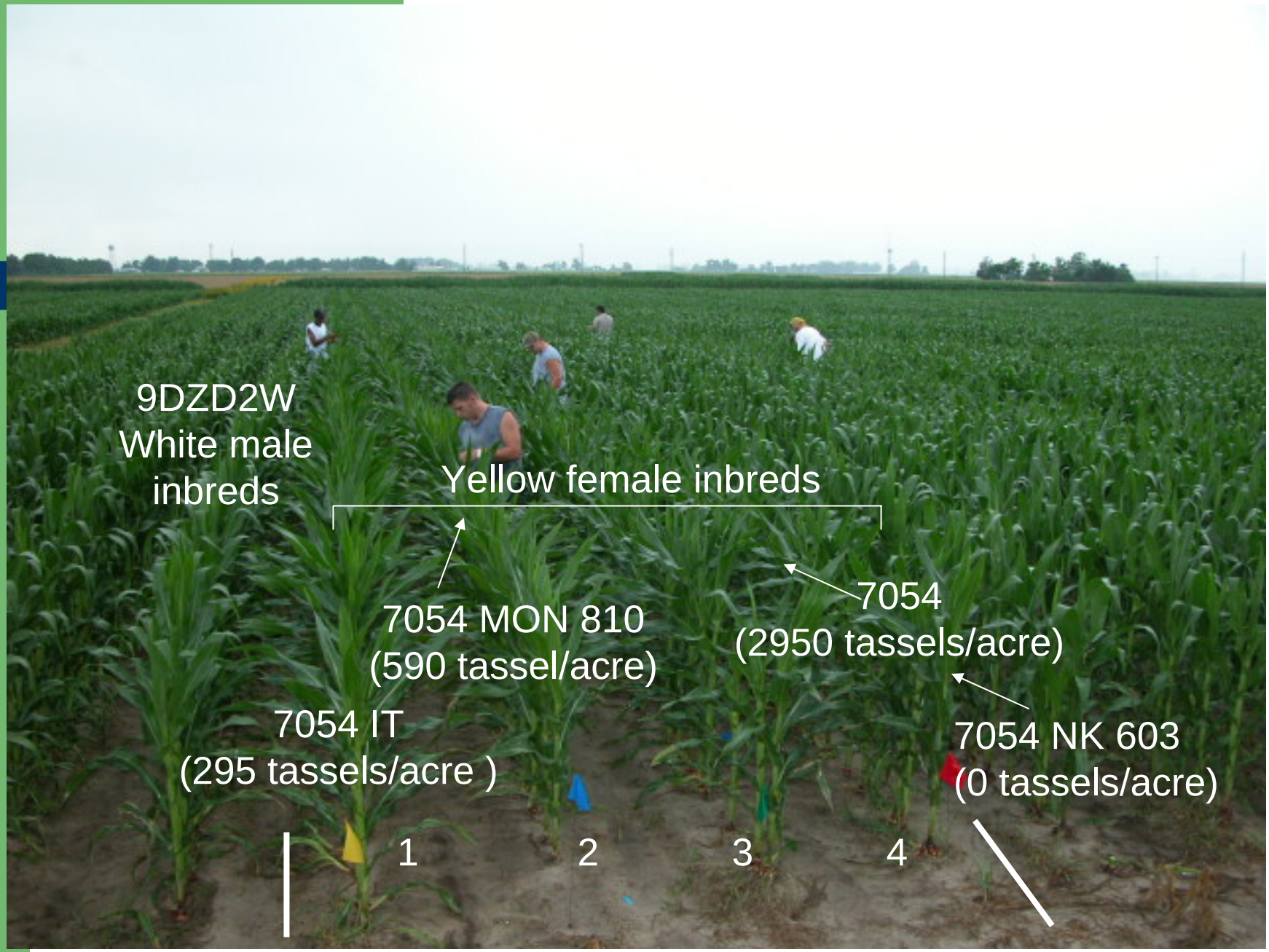
Treatments

- Yellow inbred female plants intentionally missed at rates of 0, 295, 590 and 2950 tassels/acre.
- White hybrid trap plots isolated 660 and 990 feet from female pollen blocks.

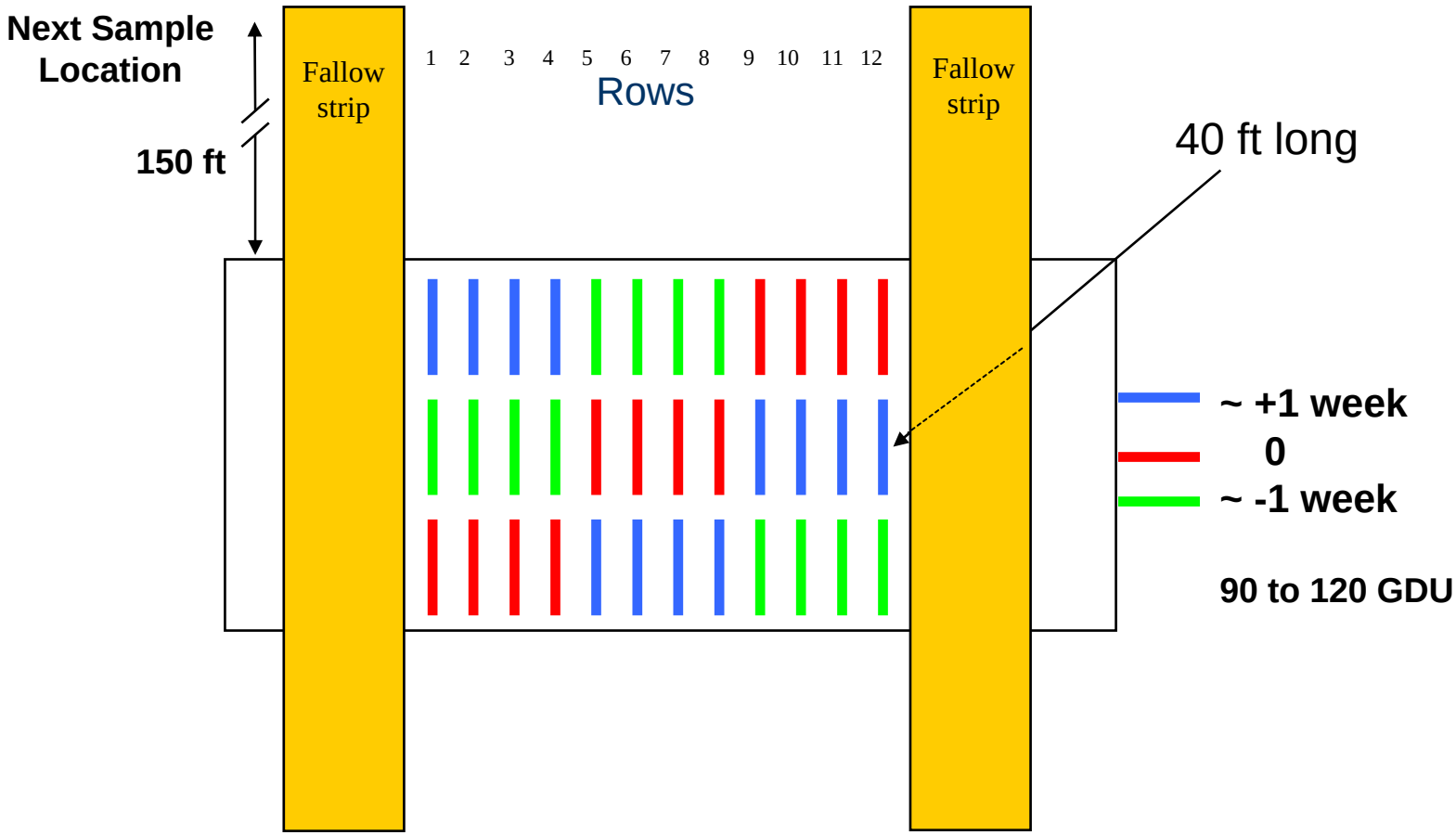

Watkins Farm
Hayti, Missouri
N
White trap plots
990 ft
660 ft
12 rows
male sterile
border
10- acre
pollen
block

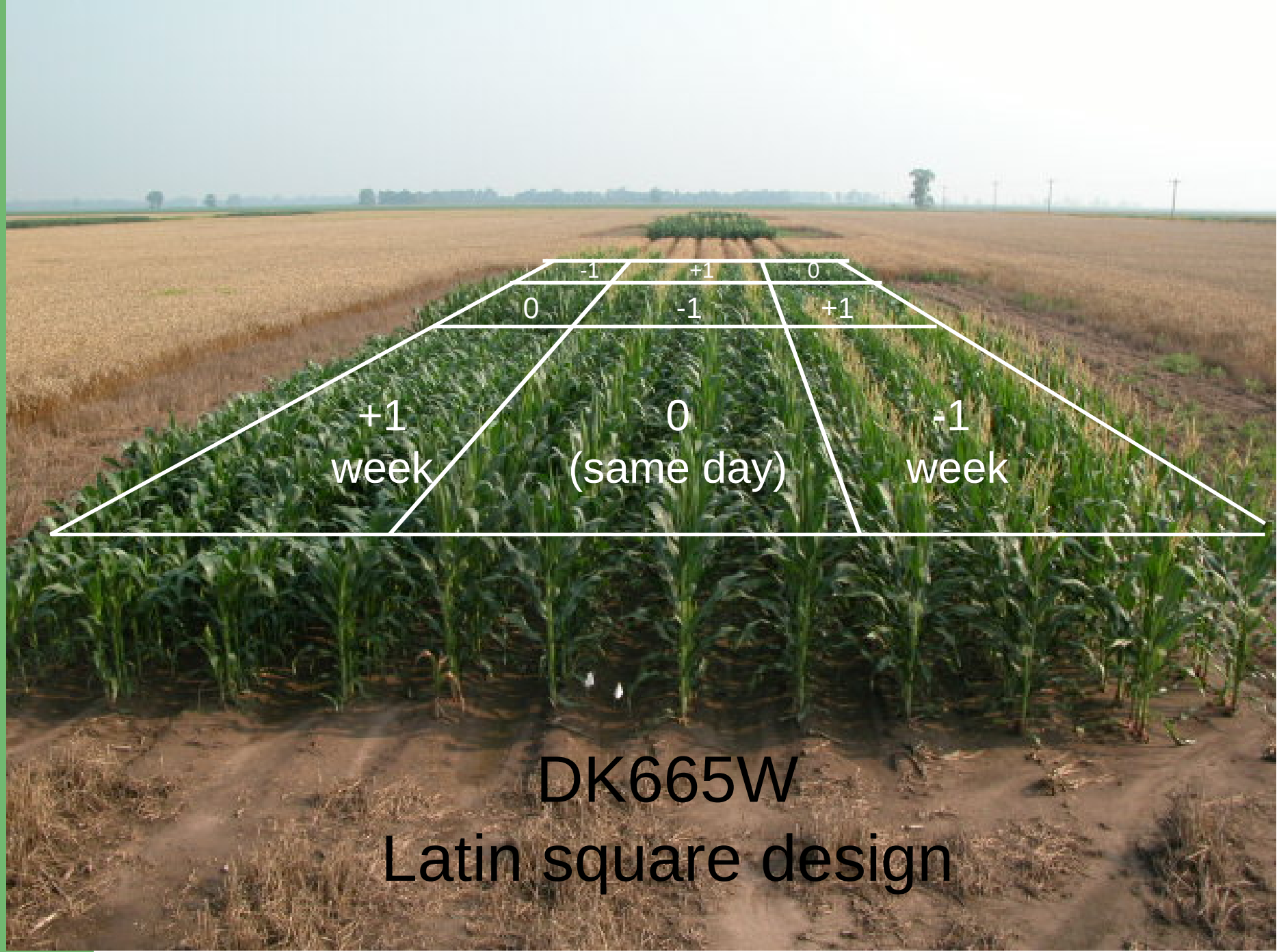
Yellow 7054 Isoline series




9DZD2W
White male
inbreds
Yellow female inbreds
7054 MON 810
(590 tassel/acre)
7054
(2950 tassels/acre)
7054 IT
(295 tassels/acre)
7054 NK 603
(0 tassels/acre)
1
2
3
4

Trap Plots- Latin Square

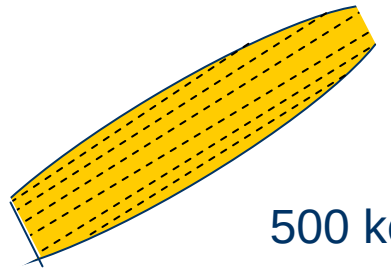




DK665W
Latin square design

Inspected 70 ears per plot

Perspective



500 kernel ear

- 0.1% is 1 kernel/2 ears= 1/1000
- 0.01% is 1 kernel/20 ears= 1/10,000
- 0.001% is 1 kernel/200 ears= 1/100,000
- 0.0001% is 1 kernel/2000 ears= 1/1,000,000

68,565 ears (34 million kernels) examined in study.

2001 Best Nicks

Location	Planting Date	Gene Flow %	Sampled
Clarkton	-1 week	0.0023 b	no
	0	0.0097 ab	no
	+1 week	0.0261 a	yes
Hayti	-1 week	0.0004 b	no
	0	0.0052 ab	no
	+1 week	0.0188 a	yes
Wardell	-1 week	0.0007 a	no
	0	0.0202 a	yes
	+1 week	0.0065 a	no

2002 Best Nicks

Site	Planting Date	Gene flow %	Sampled
Clarkton	-1 week	0.0075 a	yes
	0	0.0075 a	yes
	+1 week	0.0029 a	no
Hayti	-1 week	0.0291 a	yes
	0	0.0323 a	yes
	+1 week	0.0031 b	no
Wardell	-1 week	0.0012 a	yes
	0	0.0022 a	yes
	+1 week	0.0006 a	no

Event-specific PCR

2001 Bt and RR by gel electrophoresis

IT by end-point taqman

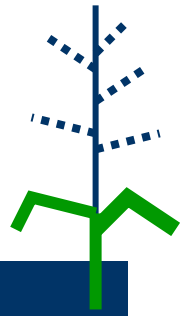
Spatial Isolation

Distance	Gene flow %
660 ft	0.0042
990 ft	0.0027

Averaged across Clarkton and Hayti 2001-2002.

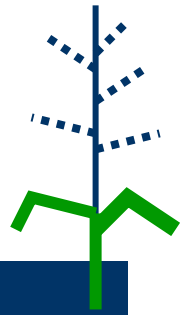
Significant interactions found distance and other factors.

Hayti 2001 isolated 990 feet



Direction	Tassels/a	Gene Flow %
North	2950	0.0100 a
	590	0.0011 b
	295	0.0002 c
	0	Not detected c
South	2950	0.0066 a
	590	0.0029 b
	295	0.0004 c
	0	Not detected c

Hayti 2002 isolated 990 feet

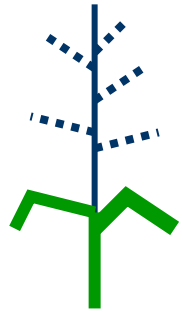


Direction	Tassels/a	Gene Flow %
North	2950	0.0144 a
	590	0.0006 b
	295	<0.0001 c
	0	Not detected c
South	2950	0.0028 a
	590	0.0004 b
	295	0.0002 b
	0	Not detected b

Clarkton 2001 isolated 990 feet

Direction	Tassels/a	Gene Flow %
North	2950	0.0234 a
	590	0.0020 b
	295	0.0013 c
	0	Not detected c
South	2950	0.0098 a
	590	0.0004 b
	295	0.0002 c
	0	Not detected b

Clarkton 2002 isolated 990 feet



Direction	Tassels/a	Gene Flow %
North	2950	0.0100 a
	590	0.0014 b
	295	0.0003 bc
	0	Not detected c
South	2950	0.0031 a
	590	Not detected b
	295	Not detected b
	0	Not detected b

One positive for 0 treatment

- Clarkton 2001 N at 660 feet isolation
- One out of 711,760 kernels at dist/dir
- Human error ???
  - Missed tassel from late tiller or early plant
  - Seed lot contamination in other isoline
  - Female row flagged with wrong color

Summary

- Complete and timely detasseling is necessary for proposed PMP maize pollen confinement system.
- Detasseling layered with highest spatial isolation (990 ft) showed no detectable gene flow.
- Adding male sterility would probably further reduce the likelihood of gene flow.